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Atty. Dkt. No. 071949-2104 (Formerly 244/121)
Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: BUECHLER et al.

Title: NOVEL METHODS FOR THE
ASSAY OF TROPONIN I AND T
AND COMPLEXES OF
TROPONIN I AND T AND
SELECTION OF ANTIBODIES
FOR USE IN IMMUNOASSAYS

Appl. No.: 09/349,194

Filing Date: July 7, 1999

Examiner: Gail Gabel

Art Unit: 1641

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| <p>CERTIFICATE OF MAILING</p> <p>I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date below.</p> <p><u>Line Gauthier</u> (Printed Name)</p> <p><u>Line Gauthier</u> (Signature)</p> <p><u>December 13, 2002</u> (Date of Deposit)</p> |
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DECLARATION OF KENNETH F. BUECHLER

Assistant Commissioner for Patents
Washington, D.C. 20231

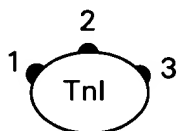
Sir:

I, Kenneth F. Buechler, declare that:

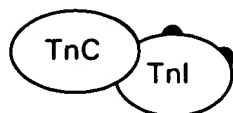
1. I earned a Ph.D. in 1981 from the Department of Biochemistry, Indiana University. I have been engaged in research involving diagnostic assays for 17 years. A copy of my curriculum vitae is attached hereto as Appendix A. I am currently employed as Vice President, Research and Development, at BIOSITE, Inc., 11030 Roselle Street, San Diego, CA 92121. I am an inventor in the above-captioned patent application.
2. I have reviewed the above-captioned patent application, and the most recent office action received in the patent application.

3. It is my opinion that the skilled artisan could perform the methods as presently claimed using only methods that are both routine and well known in the art. In particular, methods for identifying antibodies that specifically bind to both free and complexed cardiac specific isoforms of troponin or, conversely, antibodies that distinguish between free and complexed cardiac specific isoforms of troponin, are described in detail in the present specification, and can be performed using only methods that are both well known and routine in the art.

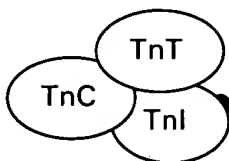
4. Taking cardiac specific troponin I ("TnI") as an example, the following drawings describes why it is possible for the skilled artisan to identify such antibodies. Troponin I contains certain antigenic sites that are "cardiac specific," meaning they are not present in non-cardiac forms of troponin I (for example, troponin I from skeletal muscle). These regions may be present as shown in red in this schematic drawing:



5. Binding of troponin C ("TnC") may obscure one or more of these cardiac specific regions so that it is not accessible to antibodies; however, one or more other cardiac specific regions may remain accessible. Antibodies may thus be selected that are specific for the free form of TnI (e.g., directed to site 1), or that bind both the free and binary complexed forms of TnI (e.g., directed to site 2 or 3):



6. Similarly, troponin T ("TnT") may also obscure one or more of these cardiac specific regions so that it is not accessible to antibodies; however, one or more other cardiac specific regions may remain accessible. Antibodies may thus be selected that bind the free and binary and ternary complexed forms of TnI (e.g., directed to site 3):



7. Thus, an assay system may utilize an antibody formed by pooling multiple antibody types in a cocktail, some of which recognize troponin only in complexes, some of which recognize only free troponin; alternatively, an assay system may utilize an antibody formed of one antibody type that recognizes a site that is available in both free and complexed troponin. This point is made clearly in the present application. For example, on page 24, lines 21-29, the specification states that "[t]he immunoassay can be formulated with a cocktail of antibodies to bind all the troponin complexes and the free troponin I and T. Alternatively, the immunoassay can be formulated with specific antibodies that recognize epitopes of the troponin I and T in the complexes and also the unbound troponin I and T."

8. The specification continues by providing examples of just such antibodies. For example, in Example 10, on page 63, lines 20-26, the specification notes that exemplary assays can be formulated to identify both free troponin I and binary troponin I complex, or that recognize only the free form. Similarly, in Example 15, on page 72, lines 12-27, the specification notes that

exemplary assays can be formulated to identify both free troponin I and ternary troponin I complex, or that recognize only the free form, or that recognize only the ternary complex.

9. It should be noted that the phrase "an antibody" recited in the claims would not be understood by the skilled artisan to imply a single molecule of antibody, but rather to refer to the population of antibody used in the particular assay.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Capital Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Sept 11, 2002
Date

Kenneth F. Buechler
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Education and Professional Experience:

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| High School, Indianapolis, IN | May 1971 |
| B. Sc., Chemistry, Indiana University Bloomington, IN | May 1975 |
| M. Sc., Biochemistry, Indiana University School of Medicine, Indianapolis, IN | Sept. 1976 – Oct. 1978 |
| Research Associate, Indiana University School of Medicine, Indianapolis, IN | Oct. 1978 – Feb. 1979 |
| Ph.D. Biochemistry, Indiana University School of Medicine, Indianapolis, IN | Mar. 1979 – June 1981 |
| Predoctoral Research Fellow, Laboratory of Veterinary Biochemistry, State University Of Utrecht, Utrecht, The Netherlands | July 1980 – Dec. 1980 |
| Postdoctoral Research Fellow, Laboratory of Veterinary Biochemistry, State University Of Utrecht, Utrecht, The Netherlands | July 1981 – Dec. 1981 |
| Postdoctoral Research Fellow, Graduate Department of Biochemistry, Brandeis University, Waltham, MA | Jan. 1982 – Feb. 1985 |
| Postdoctoral Research Fellow, Departamento de Bioquímica, Facultad de Medicina UAM, Madrid, Spain | May 1984 – July 1984 |

Research Scientist, Hybritech, Incorporated
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Mar. 1985 – Mar. 1986

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PUBLICATIONS

KENNETH F. BUECHLER

1. Wu, Alan H. B., Feng, Yue-Jin; Moore, Robert; Apple, Fred S.; McPherson, Paul H.; Buechler, Kenneth F.; Bodor, Geza.; Characterization of Cardiac Troponin Subunit Release into Serum after Acute Myocardial Infarction and Comparison of Assays for Troponin T and I, Clin. Chem., (1998), 44(6), 1198-1208.
2. Buechler, K. F.; Moi, S., Noar, B., McGrath, D., Villela, J., Clancy, M., Shenhav, A., Colleymore, A., Valkirs, G.; Simultaneous Detection of Seven Drugs of Abuse by the Triage Panel for Drugs of Abuse, Clin. Chem., (1992), 38(9), 1678-84.
3. Buechler, K. F.; Moi, S., Noar, B., McGrath, D., Villela, J., Clancy, M., Shenhav, A., Colleymore, A., Valkirs, G.; Simultaneous Detection of Seven Drugs of Abuse by the Triage Panel for Drugs of Abuse, Clin. Chem., (1992), 38(9), 1678-84.
4. Buechler, Kenneth, F., Lowenstein, John, F.; The Involvement of Carnitine Intermediates in Peroxisomal Fatty Acid Oxidation: A Study with 2- Bromofatty Acids, Arch. Biochem. Biophys. (1990), 281 (2), 233-8.
5. Buechler, K. F., Beynen, A. C., and Geelen, M. J. H.; Studies on the Assay, Activity And Sedimentation Behavior of Acetyl CoA Carboxylase from Isolated Hepatocytes Incubated with Insulin or Glucagon, (1984), Biochem. J. 221, 869-874.
6. Buechler, K. F., and Gibson, D. M.; Guanosine Triphosphate and Colchicine Affect the Activity and the Polymeric State of Acetyl CoA Carboxylase (1984) Arch. Biochem. Biophys. 233, 698-707.
7. Beynen, A. C., Buechler, K. F., Van Der Molen, A. J., and Geelen, M. J. H.; The Effects of Lactate and Acetate on Fatty Acid and Cholesterol Biosynthesis by Isolated Rat Hepatocytes, (1982) Int. J. Biochem. 14, 165-169.
8. Beynen, A. C., Buechler, K. F., Van der Molen, A. J. and Geelen, M. J. H.; Inhibition of Hepatic Lipogenesis by Salicylate (1982) Toxicology, 24, 33-43.

9. Beynen, A. C., Buechler, K. F., Van der Molen, A. J., and Geelen, M. J. H.; Inhibition of Lipogenesis in Isolated Hepatocytes by 3-Amino-1,2,4-triazole (1981) Toxicology, 22, 171-178.
10. Buechler, K.F., Geelen, M. J. H., and Beynen, A.C.; Rapid and Simple Method Simple Method for Measuring the Linearity of Sucrose Gradients, (1981) Fresenius Z. Anal. Chem. 307, 413-414.
11. Buechler, K. F., and Rhoades, R. A.; De novo Fatty Acid Synthesis in the Perfused Rat Lung: Incorporation of Palmitate into Phospholipids (1981) Biochim. Biophys. Acta 665, 393-398.
12. Buechler, K. F., and Rhoades, R. A.; Fatty Acid Synthesis in the Perfused Rat Lung (1980) Biochim. Biophys. Acta, 619, 186.

PATENTS

KENNETH F. BUECHLER

1. Buechler, Kenneth F.; Diagnostic Devices Method and Apparatus for the Controlled Movement of Reagents Without Membranes; US6,271,040, August 7, 2001.
2. Buechler, Kenneth F.; Noar, Joseph B.; Tadesse, Lema; Fluorescence Energy Transfer in Intramolecular Energy Transfer in Particles Using Novel Compounds; US6,251,687, June 26, 2001.
3. Buechler, Kenneth F.; Noar, Joseph B.; Tadesse, Lema; Fluorescence Energy Transfer Particles; US6,238,931, May 29, 2001.
4. Buechler, Kenneth F.; Anderberg, Joseph M.; McPherson, Paul H.; Methods for Monitoring the Status of Assay and Immunoassays; US6,194,222 B1, February 27, 2001.
5. Buechler, Kenneth F.; McPherson, Paul H.; Methods for the Assay of Troponin I and T and Complexes of Troponin I and T and Selection of Antibodies for use in Immunoassays; US6,174,686 B1, January 16, 2001.
6. Buechler, Kenneth F.; McPherson, Paul H.; Methods for the Recovery and Measurement of Troponin Complexes; US6,156,521, December 5, 2000.
7. Buechler, Kenneth F.; Diagnostic Devices and Apparatus for the Controlled Movement of Regents Without Membranes; US6,156,270, December 5, 2000.
8. Buechler, Kenneth F.; Non-Porous Diagnostic Devices for the Controlled Movement of Reagents; US6,143,576, November 7, 2000.
9. Buechler, Kenneth F.; Anderberg, Joseph; McPherson, Paul H.; Methods for Monitoring the Status of Assays and Immunoassays; EP1046122, October 25, 2000.

10. Buechler, Kenneth F.; Devices Comprising Multiple Capillarity Inducing Surfaces; US6,113,855, September 5, 2000.
11. Buechler, Kenneth F.; Briggs, Jason C.; Rongey, Scott H.; A Lysis Chamber for Use in an Assay Device Particularly in Blood Analysis; US6,106,779, August 22, 2000.
12. Buechler, Kenneth F.; Anderberg, Joseph; McPherson, Paul H.; Media Carrier for an Assay Device; US6,074,616, June 13, 2000.
13. Buechler, Kenneth F.; Preparation of Novel Propoxyphene Derivatives and Protein and Polypeptide Propoxyphene Derivative Conjugates and Labels; US6,037,455, March 14, 2000.
14. Buechler, Kenneth F.; Rapid Evaluation of the Ratio of Biological Molecules; EP0983509, March 8, 2000.
15. Buechler, Kenneth F.; Non-Porous Diagnostic Devices for the Controlled Movement of Reagents; US6,019,944, February 1, 2000.
16. Buechler, Kenneth F.; McPherson, Paul H.; Sundquist, Alfred R.; Novel Compositions and Methods for Inhibiting Light-Induced Inactivation of Biological Reagents in Fluorescent Conjugates; EP0972194, January 19, 2000.
17. Buechler, Kenneth F.; Noar, Joseph B.; Tadesse, Lema; Fluorescence Energy Transfer and Intramolecular Energy Transfer in Particles Using Novel Compounds; EP0972183, January 19, 2000.
18. Valkirs, Gunars E.; Buechler, Kenneth F.; Antibodies to Ligand Analogues and Their Utility in Ligand-Receptor Assays; US5,985,579, November 16, 1999.
19. Buechler, Kenneth F.; McPherson, Paul H.; Methods for Improving the Recovery of Troponin I and T in Membranes, Filters and Vessels; EP0946879, October 6, 1999.
20. Buechler, Kenneth F.; Diagnostic for Determining the Time of A Heart Attack; US5,947,124, September 7, 1999.
21. Buechler, Kenneth F.; Devices Comprising Multiple Capillarity Inducing Surfaces; EP0938659, September 1, 1999.
22. Buechler, Kenneth F.; Non-competitive Threshold Ligand-receptor Assays; US5,939,272, August 17, 1999.
23. Buechler, Kenneth F.; Anderberg, Joseph M.; McPherson, Paul H.; Media Carrier for an Assay Device; WO9935718, July 15, 1999.
24. Buechler, Kenneth F.; Anderberg, Joseph M.; McPherson, Paul H.; Methods for Monitoring the Status of Assays; WO9935602, July 15, 1999.

25. Buechler, Kenneth F.; Anderberg, Joseph M.; McPherson, Paul H.; Immunoassay Fluorometer; WO9935486, July 15, 1999.
26. Nowakowski, Mark R.; Buechler, Kenneth F.; Anderson, Richard R.; Valkirs, Gunars E.; Assay Devices Comprising a Porous Capture Membrane in Fluid-withdrawing Contact with a Nonabsorbent Capillary Network; US5,922,615, July 13, 1999.
27. Buechler, Kenneth F.; McPherson, Paul H.; Methods for the Recovery and Measurement of Troponin Complexes; WO9932888, July 01, 1999.
28. Buechler, Kenneth F.; Batt, Richard R.; Devices Incorporating Filters for Filtering Fluid Samples; EP0920356, June 9, 1999.
29. Buechler, Kenneth F.; Briggs, Jason Christopher; Rongey, Scott Harold; A Lysis Chamber for Use in an Assay Device; WO9918433, April 15, 1999.
30. Buechler, Kenneth F.; Diagnostic Devices and Apparatus for the Controlled Movement of Reagents without Membranes; US5,885,527, March 23, 1999.
31. Buechler, Kenneth F.; Valkirs, Gunars E.; Anderson, Richard R.; Non-competitive Threshold Ligand-receptor Assays; WO9857172, December 17, 1998.
32. Buechler, Kenneth F.; Rapid Evaluation of the Ratio of Biological Molecules; WO9852041, November 19, 1998.
33. Buechler, Kenneth F.; McPherson, Paul H.; Sundquist, Alfred R.; Novel Compositions and Methods for Inhibiting Light-induced Inactivation of Biological Reagents; WO9845705, October 15, 1998.
34. Buechler, Kenneth F.; Noar, Joseph B.; Tadesse, Lema; Hybrid Phthalocyanine derivatives and Their Uses; US5,824,799, October 20, 1998.
35. Buechler, Kenneth F.; Diagnostic Devices and Apparatus for the Controlled Movement of Reagents Without Membranes; WO9843739, October 8, 1998.
36. Buechler, Kenneth F.; Methods for the Assay of Troponin I and T and Selection of Antibodies for use in Immunoassays; US5,795,725, August 18, 1998.
37. Buechler, Kenneth F.; McPherson, Paul H.; Method for Improving the Recovery of Troponin I and T; WO9827435, June 25, 1998.
38. Buechler, Kenneth F.; Noar, Joseph B.; Novel Derivatives of Tricyclic Antidepressants and Protein and Polypeptide Tricyclic Antidepressant Derivative Conjugates and Labels; EP0846126, June 10, 1998.
39. Buechler, Kenneth F.; Noar, Joseph B.; Tadesse, Lema; Fluorescence Energy Transfer and Intramolecular Energy Transfer in Particles Using Novel Compounds; US5,763,189, June 9, 1998.

40. Buechler, Kenneth F.; Devices Comprising Multiple Capillarity Inducing Surfaces; WO9821563, May 22, 1998.
41. Buechler, Kenneth F.; Novel Methadone Derivatives and Protein and Polypeptide Methadone Derivative Conjugates and Labels; EP0827502, March 11, 1998.
42. Buechler, Kenneth F.; Batt, Richard Roger; Devices Incorporating Filters for Filtering Fluid Samples; WO9808606, March 5, 1998.
43. Buechler, Kenneth F.; McPherson, Paul H.; Novel Methods for the Assay of Troponin I and T and Selection of Antibodies for use in Immunoassays; EP0821794, February 4, 1998.
44. Buechler, Kenneth F.; Noar, Joseph, B.; Tadessee, Lema; Fluorescence Energy Transfer and Intramolecular Energy Transfer in Particles Using Novel Compounds; EP0820489, January 28, 1998.
45. Buechler, Kenneth F.; Fluorescence Energy Transfer and Intramolecular Energy Transfer in Particles Using Novel Compounds; EP0670041, January 28, 1998.
46. Buechler, Kenneth F.; Novel Methadone Derivatives and Protein and Polypeptide Methadone Derivative Conjugates and Labels; US5,710,256, January 20, 1998.
47. Buechler, Kenneth F.; Valkirs, Gunars E.; Threshold Ligand-Receptor Assay; US5,679,526, October 21, 1997.
48. Buechler, Kenneth F.; Novel Opiate Derivatives and Protein and Polypeptide Opiate Derivative Conjugates and Labels; US5,610,283, March 11, 1997.
49. Buechler, Kenneth Francis; Noar, Joseph Barry; Preparation of Tricyclic Antidepressant Conjugates useful in immunoassays; WO9708192, March 6, 1997.
50. Buechler, Kenneth F.; Noar, Joseph B.; Tedesse, Lema; Hybrid Phthalocyanine Derivatives And their Uses; WO9629367, September 26, 1996.
51. Buechler, Kenneth F.; Anderson, Richard R.; Lee, Theodore T.; Valkirs, Gunars E.; Crosstalk Inhibitors and Their Uses; US5,525,524, June 11, 1996.
52. Valkirs, Gunars Edwin; Buechler, Kenneth F.; Antibodies to Complexes of Ligand Receptors and Ligands and their Utility in Ligand-receptor Assays; US5,480,792, January 2, 1996.
53. 20. Buechler, Kenneth F.; Methadone Derivatives and Protein and Polypeptide Methadone Derivative Conjugates and Labels; WO9631496, October 10, 1996.
54. 21. Buechler, Kenneth F.; McPherson, Paul H.; Novel Methods for the Assay of Troponin I and T and Complexes of Troponin I and T and Selection of Antibodies for Use in Immunoassays; WO9633415, October 24, 1996.
55. Buechler, Kenneth F.; Noar, Joseph B.; Si, Shi; Amphetamine Derivatives and Protein and

Polypeptide Amphetamine Derivative Conjugates and Labels; US5,470,997, November 28, 1995.

56. Buechler, Kenneth F.; Diagnostic Devices for the Controlled Movement of Reagents without Membranes; US5,458,852, October 17, 1995.
57. Buechler, Kenneth F.; Barbiturate Derivatives and Protein and Polypeptide Barbiturate Derivative Conjugates and Labels; US5,414,085, May 9, 1995.
58. Buechler, Kenneth Francis; Noar, Joseph Barry; Tadessee, Lema; Fluorescence Energy Transfer in Particles Using Novel Compounds; WO9508772, March 30, 1995.
59. Buechler, Kenneth F.; Novel Opiate Derivatives and Protein and Polypeptide Opiate Derivative Conjugates and Labels; EP0644884, March 29, 1995.
60. Buechler, Kenneth F.; Opiate Derivatives and Protein and Polypeptide Opiate Derivative Conjugates and Labels; EP0635019, January 25, 1995.
61. Buechler, Kenneth F.; Novel Propoxyphene Derivatives and Protein and Polypeptide Propoxyphene Derivative Conjugates and Labels' EP0638067, January 21, 1995.
62. Buechler, Kenneth F.; Phencyclidine Derivatives and Protein and Polypeptide Phencyclidine Derivative Conjugates and Labels; US5,331,109, July 19, 1994.
63. Buechler, Kenneth Francis; Preparation of Novel Propoxyphene Derivative Conjugates and Labels; WO9411405, May 26, 1994.
64. Buechler, Kenneth F.; Diagnostic Devices and Apparatus for the Controlled Movement of Reagents Without Membranes; EP0596104, May 11, 1994.
65. Buechler, Kenneth F.; Moi, S.; Tetrahydrocannabinol Derivatives and Protein and Polypeptide Tetrahydrocannabinol Derivative Conjugates and Labels; US5,302,703, April 12, 1994.
66. Buechler, Kenneth F.; Noar, Joseph B.; Benzodiazepine Derivatives; US5,302,715, April 12, 1994.
67. Buechler, Kenneth Francis; Anderson, Richard Ray; Lee, Theodore Tsan-Tsung; Crosstalk Inhibitors and Their Uses; EP0585310, March 9, 1994.
68. Buechler, Kenneth F.; Novel Cocaine Derivatives and Protein and Polypeptide Cocaine Derivative Conjugates and Labels; EP0575581, December 29, 1993.
69. Buechler, Kenneth Francis; Preparation of Functionalized Morphine Derivatives as Hapten Conjugate Intermediates; WO9320079, October 14, 1993.
70. Buechler, Kenneth Francis; Phencyclidine Derivatives for Preparation of Protein and Polypeptide-phencyclidine Derivative Conjugates; WO9320049, October 14, 1993.
71. Buechler, Kenneth Francis; Noar, Joseph Barry; Moi, Si Shi; Novel Amphetamine

Derivative Conjugates and Labels; WO9320048; October 14, 1993.

72. Buechler, Kenneth Francis; Preparation of Tetrahydrocannabinol Derivatives for Covalent Preparation of Tetrahydrocannabinol Derivatives for Covalent Attachments to Proteins or Polypeptides; US5,237,057, August 17, 1993.
73. Buechler, Kenneth F.; Cocaine Derivatives; US5,233,042, August 3, 1993.
74. Buechler, Kenneth Francis; Cocaine Derivatives and Cocaine Derivatives Conjugates With Polypeptides and Label for Immunoassays; WO9312111; June 24, 1993.
75. Buechler, Kenneth Francis; Conjugate of Polymeric Dye and Biospecific Antibody for Spectrometric Immunoassay; WO9220746, November 26, 1992.
76. Buechler, Kenneth Francis; Benzodiazepine Derivatives and Protein and Polypeptide Conjugates Thereof; WO9320067, October 14, 1992.
77. Valkirs, Gunars Edwin; Buechler, Kenneth F.; Antibodies to Ligand Analogs and their Utility in Ligand-Receptor Assays; US5,143,852, September 1, 1992.
78. Buechler, Kenneth F.; Valkirs, Gunars E.; Antibodies to Complexes of Ligand Receptors and Ligands and Their Utility in Ligand-Receptor Assays; EP0475784, March 18, 1992.
79. Buechler, Kenneth, F.; Valkirs, Gunars E.; Anderson, Richard Ray; Threshold Ligand-Receptor Assay; US5,089,391; February 18, 1992.
80. 40. Nowakowski, Mark Ronald; Buechler, Kenneth Francis; Valkirs, Gunars Edward; Bioassay Device with Non-Absorbent Textured Capillary Surface; WO9113998; September 19, 1991.
81. Nowakowski, Mark Ronald; Buechler, Kenneth Francis; Valkirs, Gunars Edward; Anderson, Richard Ray; Device for Ligand-receptor Methods; EP0447154, September 18, 1991.
82. Buechler, Kenneth Francis; Valkirs, Gunars E.; Anderson, Richard Ray; Threshold Ligand-Receptor Assay; US5,028,535, July 2, 1991.
83. Buechler, Kenneth F.; Valkirs, Gunars E.; Anderson, Richard R.; Treshold Ligand-Receptor Assay; EP0378391, July 18, 1990.